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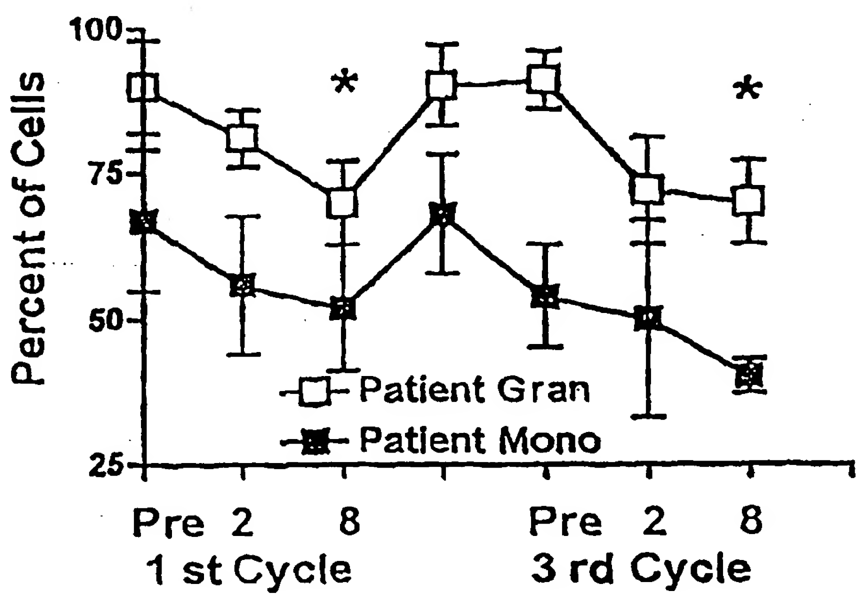
(54) Title: METHOD FOR THE PREVENTION AND TREATMENT OF A TYPE I HYPERSENSITIVITY DISORDER

(57) Abstract

Administration of Rh antibodies to an animal is effective for reducing the intensity, duration or frequency of asthmatic exacerbations, and of other type-I hypersensitivity disorders. Compositions thereof are claimed and so are their uses to prevent such disorders. Treatment with Rh antibodies in said mammal can also reduce the dosage of concomitant or other medications required by the animal for the control of this illness.



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Title: Method for the Preventi n and Treatment of a Type I Hypersensitivity Disorder

FIELD OF INVENTION

The present invention relates to methods and compositions for treating a type I hypersensitivity disorder using Rh antibodies. In particular, Rh_o(D) immune globulin reduces the duration and/or intensity of exacerbations in asthmatic patients and reduces the dose requirement of concomitant or other therapeutics such as corticosteroids in asthmatic patients. Rh_o(D) immune globulin is also useful in preventing the onset and reduce the frequency of exacerbations or episodes in asthmatic patients.

BACKGROUND OF THE INVENTION

Hypersensitivity generally refers to an inappropriate or exaggerated immune response to a particular antigen. There are four major classes of allergic or hypersensitivity reactions. Type I hypersensitivity results when an immunoglobulin E (IgE) response is mounted against an innocuous antigen causing mast cells to release mediators which in turn cause inflammation. Many conditions result from a type I hypersensitivity response including allergies, eczema, hayfever, urticaria, atopic dermatitis and asthma (see also Gell-Coombs classification: Coombs, R.R.A. and Gell, P.G.H.: Classification of allergic reactions responsible for clinical hypersensitivity and disease. *In* Clinical Aspects of Immunology, eds. Gell, P.G.H., Coombs, R.R.A. and Lachmann, P.J., Blackwell Scientific Publications, Oxford, 1975).

Asthma is a chronic inflammatory illness characterized predominantly by reversible airway obstruction and airway hyperresponsiveness. The major clinical symptoms of asthma include recurrent episodes of wheezing, breathlessness, chest tightness and coughing. Asthma is a common illness affecting 5-10% of the population, and it is associated with substantial mortality which is increasing in certain countries (Sears, M.R. and Taylor, D.R., *Drug Safety* 11: 259-283, 1994; Page, C.P., *J. Asthma* 30: 155-164, 1993; Weiss, K.B., *JAMA* 264: 1683-1687, 1990; Gergen, P.J. and Weiss, K.B., *JAMA* 264: 1688-1692, 1990).

The pathogenesis of asthma is currently thought to be due, in large, to inflammatory-based changes in the airway wall and to airway smooth muscle dysfunction (Hogg, J.C., *APMIS* 105: 735-745, 1997). The basic mechanism underlying airway narrowing or broncoconstriction in asthma is typified by a type I immediate hypersensitivity illness and is believed to involve an activation of mast cells which release a large number of inflammatory mediators such as histamine, leucotrienes, cytokines and proteases. These mediators exert potent effects to produce airway vasodilatation and increase vasopermeability. Influx of inflammatory cells including granulocytes (such as eosinophils), monocytes (precursors of macrophages) and lymphocytes into the brochial tissue is also increased and these cells in turn secrete additional mediators of inflammation which further enhance brochoconstriction, mucus secretion and tissue inflammation (Serafin, W.E. In *Goodman and Gilman's The*

Pharmacological Basis of Therapeutics, Chap. 28, p. 659-682, 1996; Priel, I.E., *Med. Law* 12: 351-361, 1993; Hughes, J.M., et. al., *Clin. Exp. Allergy* 23: 251-256, 1993). Inappropriate immune responses to airborne antigens by the respiratory T-lymphocyte system have also been attributed to play a part in the immunopathology of asthma (Holt, P.G. et al., *Eur. Respir. J.* 4 Suppl. 13: 6s-15s, 1991; Jansen, H.T. et al., *Eur. Respir. J.* 4 Suppl. 13: 3s-5s, 1991; Holgate, S.T., *Eur. Respir. J.* 6: 1507-1520, 1993). T-lymphocytes overproduce cytokines such as interleukins to cause and maintain excessive infiltration and to upregulate function of eosinophils and mast cells. Mast cells are primarily responsible for the secretion of the vasoactive amine histamine while eosinophils have direct cytotoxic actions in the bronchial epithelium and are the major cause of bronchial tissue damage in asthma (Karlen, S. et. al., *Int. Rev. Immunol.* 16: 227-247, 1998; Tonnel, A.B. et. al., *Rev. Prat.* 42: 2399-2404, 1992).

Classification or categorization of asthma can be based upon disease etiology or severity (Fabbri, L.M. et al., In *Allergy and Allergic Diseases*, Chap. 87, p. 1347-1359, 1997). From an etiology perspective, asthma may be categorized as extrinsic (atopic) or intrinsic. Extrinsic asthma is used to define asthma occurring in atopic patients with an increased amount of IgE against common environmental allergens. It is often possible to identify the immunogens associated with the onset of such an asthmatic attack or exacerbation, but an increasing number of non-allergenic agents that can also provoke an asthmatic attack has also been found. Identified environmental agents that could instigate asthmatic exacerbations are diverse and include, but are not limited to, common allergens (e.g. proteins found in ragweed, dust mite and pet dander), environmental pollutants such as sulphur dioxide as well as various pathogens in respiratory infections such as for example bacteria and viruses.

The category of intrinsic or non-atopic asthma is used to define a group of patients for whom no extraneous or external causative agent can be identified.

A second classification system for asthma is based on the severity of the illness. The overall severity of exacerbations among asthmatic patients can vary substantially in the clinical setting, and determination of the severity of asthma is often based primarily on a combination of symptom scores and spirometry measurements (Sheffer, A.L., Publication No. 95-3659, World Health Organization and National Institute of Health, 1995). The type and amount of therapeutic intervention required to control the illness is often correlated with illness severity (see below).

A majority of asthmatic patients have a self-limited or easily manageable disease, but a subset of patients suffers from chronic, debilitating intractable respiratory difficulties. Major therapeutic treatment of asthma includes administration of bronchodilators as physiological antagonists and anti-inflammatory agents to counteract airway vasodilatation and vasopermeation.

Examples of bronchodilators used in the treatment of asthma are

beta-adrenergic receptor agonists, xanthine derivatives and anticholinergics. Beta-adrenergic receptor agonists (such as albuterol, terbutaline, pirbuterol, salbutamol, salmeterol and formoterol) provide rapid relief of acute bronchospasm and are also effective bronchoprotective agents for prophylaxis in atopic asthma. Albuterol and terbutaline are available as nebulizer solutions and metered dose inhalers for pulmonary drug delivery (about 200-400 ug three to four times per day). Xanthine derivatives such as theophylline (given orally at about 12-16 mg per kg per day), enprofylline and pentoxifylline, have longer duration of action than beta-adrenergic receptor agonists thereby providing adequate protection against both early and late phase responses to an allergen stimulus. However, xanthines are relatively weaker bronchodilators and bronchoprotectors and careful dose titration and patient monitoring are required in view of their narrow therapeutic margins. The value of anticholinergic agents in asthma therapy is to date controversial and their role in combinational therapy is under investigation.

With respect to anti-inflammatory agents, two classes of agents are available for the treatment of asthma which are namely cromolyn-based drugs and corticosteroids, or more specifically, glucocorticoids (Szefler, S.J., *Clin. Allergy* 76: 953-975, 1992). These therapeutics may be given systemically by oral administration, injection or by pulmonary delivery such as aerosols or nebulizers. The latter approach has the advantage of producing high local drug concentration in the lungs thereby improving therapeutic ratio and reducing systemic side effects.

Cromolyn-like therapeutics block both the early and late phases of pulmonary response in allergen or exercise-induced asthma and prevent the development of airway hyperresponsiveness. These compounds are relatively safe to use in all age-groups and are primarily efficacious in alleviating mild to moderate asthma. Their mechanisms of action are believed to involve the inhibition of antigen-induced bronchospasm as well as autocoid release through reduced calcium influx and altered protein phosphorylation in bronchial tissue and mast cells (Hoag and McFadden, 1991; Murphy and Kelly, 1987; Shapiro and Konig, 1985). Cromolyn sodium is usually given by inhalation as a solution or powder at about 2 mg doses four times per day while nedocromil is given also by inhalation at about 4 mg doses twice per day.

Glucocorticoids are more potent and more widely used than cromolyn compounds and systemic corticosteroid therapy has remained as the mainstay of treatment of severe asthma (McFadden, E.R., *Am. Rev. Resp. Dis.* 147: 1306-1310, 1993; Greenberger, P.A., *Chest* 101: 418S-421S, 1992). Examples of systemic glucocorticoid treatment regimens are prednisone given orally at 1-2 mg per kg patient body weight per day for 3-7 days and methylprednisolone sodium succinate given intravenously at about 1 mg per kg every 6 hours. This form of therapy has proven to be efficacious in severe acute asthma and status asthmaticus, but the benefits of asthma control must be weighed against the severe side

effects of corticosteroids. Obesity, hirsutism and Cushing-like symptoms are common drawbacks, but more severe adversities such as growth suppression, diabetes mellitus, osteoporosis and intraocular hypertension are also encountered.

To reduce the risk of adversities associated with corticosteroid therapy in patients with the more severe form of asthma, it is important to use the lowest dose possible to achieve therapeutic efficacy while minimizing the systemic drug concentration. The development of an inhalational/pulmonary route of administration for low-dose corticosteroid therapy has improved dramatically its systemic toxicity profile and has allowed corticosteroids to be used in moderate asthma. In the United States, beclomethasone diproprionate (maxima of 0.4 and 0.8 mg per day for children and adults), triamcinolone acetonide (maxima of 1.2 and 1.6 mg per day for children and adults) and flunisolide (maxima of 1.0 and 2.0 mg per day for children and adults) are available as metered dose inhalers while budesonide diproprionate (about 1 mg per kg per day) and fluticasone proprionate are available in Europe. Nevertheless, serious side effects remain prominent with high dose inhalational therapy and chronic exposure to corticosteroids can also result in progressive pulmonary toxicities such as lung fibrosis.

A second means to reduce the dosage of corticosteroids required is combinational therapy with other therapeutic agents. Certain anti-asthmatic therapeutics can complement or synergize with corticosteroids and are said to possess "corticosteroid-sparing" effects. Simultaneous or sequential administration of these agents with corticosteroids allows the reduction of the dosages of corticosteroids to achieve equivalent efficacy.

A progressive treatment schedule for asthma therapy has been proposed by Szefler, S.J. (*Clin. Allergy* 76: 953-975, 1992) which consists of four stages accounting for the severity of the illness and the corresponding aggressiveness of the treatment regimen. The first stage of the model contemplates the use of the short-acting beta-adrenergic receptor agonists to provide acute rapid relief of early onset asthmatic symptoms such as sudden episodic exacerbations precipitated by allergen exposure or respiratory infections. The next stage involves the implementation of prophylactic measures and the use of a combination of a bronchodilator (theophylline, beta-adrenergic receptor agonists) and an anti-inflammatory (cromolyn, inhalational glucocorticoid) as maintenance therapy. Symptoms in stage 3 begin to compromise the patient's quality of life. Systemic or high-dose inhalational glucocorticoid treatment is required to relieve acute exacerbations and maintenance therapy to control the frequency of exacerbations. Patients in stage 4 have deteriorating pulmonary function and suffer from frequent episodes of acute life-threatening exacerbations. They are poor-responders to conventional bronchodilator and glucocorticoid therapies and require immunomodulator therapy to counter the adverse effects of high-dose bronchodilator and glucocorticoid therapy. Patients in the latter stages can often benefit from alternative modes

of therapy which currently include only a limited choice of drug candidates: troleandomycin, methotrexate, gold, hydroxychloroquine, dapsone, cyclosporine A and immune globulin products (Ruhl, R. et al., *Allergol. Immunopathol. Madr.* 21: 53-60, 1993).

Troleandomycin (TAO) was introduced originally as a macrolide antibiotic against gram-positive bacteria and has more recently been studied for its benefits in infectious asthma (Wald, J.A. et al., *J. Allergy Clin. Immunol.* 78: 36-43, 1986; Zeiger, R.S. et al., *J. Allergy Clin. Immunol.* 66: 438-446, 1980; Fox, J.L., *Penn. Med. J.* 64: 634-635, 1961). Administration of TAO to asthmatic patients produced significant clinical improvements particularly when given conjunctive to corticosteroid therapy. A steroid-sparing effect was recognized when TAO at 250 mg per day was combined with methylprednisolone in steroid-dependent asthmatics. The underlying benefits of TAO include a reduction in sputum production and in airway inflammation. Conversely, TAO therapy has been associated with significant hepatotoxicity (contraindicated in patients with pre-existing liver disease or hypersensitivity to macrolides) and should only be used to treat very severe forms of asthma.

Methotrexate inhibits dihydrofolate reductase and has been used with some degree of success in the treatment of various immune disorders such as rheumatoid arthritis and psoriasis. More recent evidence indicates that methotrexate also reduces the serum levels of several subclasses of immune globulin and possesses anti-inflammatory properties by inhibiting C5a neutrophil chemotaxis and release of histamine and interleukin. Its usefulness in asthma therapy has been demonstrated mainly in steroid-dependent asthmatics in that patients treated with methotrexate were able to significantly reduce their corticosteroid dose requirements without changes in airway hyperresponsiveness (Dyer, P.D. et al., *J. Allergy Clin. Immunol.* 88: 208, 1991; Sorkness, C.A. et al., *J. Allergy Clin. Immunol.* 87: 298, 1991; Shiner, R.J. et al., *Lancet* 336: 137, 1990; Mullarkey, M.F. et al., *N. Engl. J. Med.* 318: 603-607, 1988; Mullarkey, M.F. et al., *Ann. Allergy* 56: 347-350, 1986). Guidelines for methotrexate therapy in the treatment of rheumatoid arthritis are applied in general to asthma therapy. It is given intramuscularly or orally in dosages of about 7-15 mg per week and the parenteral route is preferred to maximize bioavailability. Methotrexate therapy should be limited to severe steroid-requiring asthmatic patients and this drug is associated with significant side effects including gastrointestinal disturbances such as vomiting, stomatitis, diarrhea, and hepatotoxicity.

Gold salts have been used effectively in the treatment of immune disorders including rheumatoid arthritis and asthma (Foley, B. et al., *J. Allergy Clin. Immunol.* 87: 298, 1991; Bernstein, D.I. et al., *J. Allergy Clin. Immunol.* 81: 6, 1988; Muranaka, M. et al., *Ann. Allergy* 40: 132, 1978). Given weekly at dosages of about 10-100 mg as intramuscular injections, gold thiomalate produced significant clinical improvement in patients with extrinsic, but not intrinsic, asthma. More recent experience with an oral gold compound, auranofin (3 mg twice daily), suggest the ability of gold salts to decrease bronchial

hyperresponsiveness and to reduce maintenance dosages of corticosteroids (steroid-sparing effect). The mechanism of action for gold salts in asthma is not fully known but is believed to involve the inhibition of production and secretion of lyzosomal enzymes, histamine, chemokines and antibodies. Major adverse effects of gold therapy are dermatitis, proteinuria, stomatitis and diarrhea which are often reversible upon cessation of treatment.

Numerous reports have also described the successful use of miscellaneous therapeutics such as dapsone, hydroxychloroquine and cyclosporine A, in the treatment of severe corticosteroid-dependent asthma (Finnerty, N.A. and Sullivan, T.J., *J. Allergy Clin. Immunol.* 87: 297, 1991; Berlow, B.A. et al., *J. Allergy Clin. Immunol.* 87: 710-715, 1991; Charous, B.L., *Ann. Allergy* 65: 53-58, 1990). All agents were shown to alleviate asthmatic symptoms and to possess corticosteroid-sparing effects. The anti-neutrophilic effects of dapsone reduces inflammation, which may explain the efficacy of this compound. Chloroquine-based drugs may elicit their effects through stabilization of membranes and inhibition of phospholipase A_2. As an inhibitor of cytokine production, cyclosporine A is a well known immunosuppressor which may account for its anti-asthmatic effects. Data from larger scale controlled clinical studies are required to establish the usefulness and value of these agents in asthma.

The notion of immunomodulatory therapy for intervention in an immune disorder such as asthma has been known and practised for many years. Active immunotherapy with specific allergens effectively reduces symptoms of allergic diseases and decreases specific IgE levels in patients, while intravenous immune globulin (IVIG) as an immunotherapeutic has been used extensively to provide passive immunity in humans since the turn of the century.

Immune globulins (also known as immunoglobulins) are proteins produced by lymphoreticular tissues. There are 6 known classes of immune globulin: IgG, IgM, IgA, IgD, IgE and secretory IgA. IgG (also known as gamma-globulin) is the most abundant and the most therapeutically relevant class of immune globulin. The primary function of immune globulins is to specifically recognize and bind antigens through reversible bonding thereby facilitating elimination of the antigens by the immune system.

IgG is a glycoprotein of approximately 150,000 Daltons consisting of 2 "heavy" (gamma) chains and 2 "light" (kappa or gamma) chains held together by disulphide as well as weak covalent bonds. Within the IgG class, there are 4 subclasses: IgG1, IgG2, IgG3 and IgG4 comprising about 70%, 15%, 10% and 5% of total IgG in normal human serum, respectively. These subclasses possess minor antigenic differences among their "heavy" chains resulting in distinct biological actions.

There are principally two types of immune globulins that are available as therapeutic agents: standard immune serum globulin preparations for general use, and immune globulin preparations that recognize specific antigens for use in specific disorders.

Commercial examples of products in the latter category are hepatitis B immune globulin, varicella zoster immune globulin and Rh immune globulin. The primary therapeutic basis for immune globulins is passive immunity conferred to the recipient through the direct introduction of extraneous "ready-made" antibodies. The major clinical utilities of immune globulins are prophylaxis and/or treatment of antigen-associated disorders.

It is well known in the art that immune globulins may be prepared by isolation of natural immune globulins from mammalian serum or by recombinant DNA technology. In the first instance, they may be isolated using the Cohn cold ethanol fractionation method (see Huchet, J. et al., *Rev. Fr. Transfus.* 13:231, 1970; Chown, B. et al., *Can. Med. Assoc. J.* 100:1021, 1969; Barandun, S. et al., *Vox Sang.* 7: 157-174, 1962). Immune globulin prepared by this method suffers from relatively low yield and purity, and the resultant product contains aggregated polymers of the protein and cannot be administered by intravenous injection or infusion. Intravenous injection or infusion is preferred in the clinical setting due to its instant bioavailability and rapid onset of therapeutic protection as compared to other parenteral routes in which the immune globulin is partially lost due to proteolysis and/or incomplete absorption.

Improved methods have been developed to provide purer monomeric proteins and/or to substantially increase product yield. Examples of these methods include: utilizing acid treatment (see Jouvenceaux, A. et al., *Rev. Fr. Transfus.* 12 (suppl.): 341, 1969), ion-exchange chromatography *e.g.* using DEAE-Sephadex columns (see Canadian Patent number 1,168,152; Canadian Patent number 1,201,063; Cunningham, C.J. et al., *Biochem. Soc. Trans.* 8: 178, 1980; Hoppe, H.H. et al., *Vox. Sang.* 25: 308, 1973; Hoppe, H.H. et al., *Münch. Med. Wochenschr.* 109: 1749, 1967; Baumstark, J.S. et al., *Arch. Biochem.* 108:514, 1964), ultracentrifugation, or treatment with pepsin, plasmin, a sulfitolytic agent or beta-propriolactone (see U.S. Patent No. 4,160,763; Barandun, S. et al., *Monogr. Allergy* 9: 39-60, 1975; Stephan, *Vox Sang.* 28: 422-437, 1975; Wells, J.L.V. et al., *Austr. Ann. Med.* 18: 271, 1969; Baumgarten, W. et al., *Vox Sang.* 13: 84, 1967; Merler, E. et al., *Vox Sang.* 13: 102, 1967; Sgouris, J.T. et al., *Vox Sang.* 13: 71, 1967; Barandun, S. et al., *Vox Sang.* 7: 157-174, 1962; Nisonoff, A. et al., *Science* 132: 1770-1771, 1960). Immune globulin prepared by such improved processes may be administered by parenteral means including intravenous injection.

Monoclonal immune globulins can be produced using recombinant and hybridoma techniques (see Canadian Patent number 1,303,534; Canadian Patent number 1,303,533; European Patent Application 87302620.7 published as EP 239,400; European Patent Application 93102609.0 published as EP 557,897; Fletcher, A. and Thompson, A., *Transfus. Med. Rev.* 9: 314-326, 1995; Alting-Mees, M. et al., *Strat. Mol. Biol.* 3: 1-9, 1990; Huse, W.D. et al., *Science* 246: 1275-1281, 1989; Sastry, L. et al., *Proc. Natl. Acad. Sci. USA* 86: 5728-5732, 1989). Similarly, binding partners or domains may also be constructed using recombinant DNA techniques to incorporate the variable regions of a gene encoding a specific antibody

(see PCT Patent Application PCT/GB93/00605 published as WO 93/19172; PCT Patent Application PCT/GB93/02492 published as WO 94/13804; PCT Patent Application PCT/EP90/01964 published as WO 91/07492; Bird et al., *Science* 242: 423-426, 1988).

The clinical value of IVIG in asthma therapy has been postulated for many years but few controlled studies have been conducted to establish this mode of therapy. Use of IVIG in the clinical setting for this purpose remains relatively limited.

Previous reports describing the benefits of IVIG therapy include the ability of IVIG to reduce recurrent wheezing in asthmatic patients with immunodeficiencies (Smith, T.F., *J. Asthma* 26: 5-13, 1989; Smith, T.F. et al, *Monogr. Allergy* 23: 188, 1988; Page, R. et al., *J. Pediatr.* 112: 126-131, 1988) and to reduce the number/frequency of asthmatic exacerbations and improve pulmonary function (forced expiratory volume per second, thoracic gas volume, airway resistance, specific conductance) in steroid-dependent asthmatic patients without immunodeficiencies (Gelfand, E.W. et al., *Clin. Exp. Immunol.* 104 Suppl. 1: 61-66, 1996; Smiley, J.D. and Talbert, M.G., *Am. J. Med. Sci.* 308: 295-303, 1995; Silk, H.J., *J. Asthma* 31: 231-241, 1994; Schuster, A. And Wahn, V., *Infusionsther. Transfusionsmed.* 20 Suppl. 1: 141-144, 1993; Alvarez, J.M. and Szefler, S.J., *J. Asthma* 29: 3-11, 1992; Fireman, P. And Friday, G., *Clin. Rev. Allergy* 10: 135-142, 1992; Levinson, A.I., *J. Allergy Clin. Immunol.* 88: 552-554, 1991; Mazer, B.D. and Gelfand, E.W., *J. Allergy Clin. Immunol.* 87:976-983, 1991; Mazer, B.D. et al., *Clin. Immunol. Immunopathol.* 53: S156-S163, 1989). The dosages of glucocorticoid required by these patients for illness control were also significantly reduced during the IVIG treatment period. Unlike other alternative agents discussed above, side-effects observed with IVIG therapy in asthmatic patients were relatively less severe at doses of up to 2 g/kg every 4 weeks.

The exact mechanism of action underlying the action of IVIG in asthma remains to be defined. At low doses, IVIG administration may produce its effects through an increase in patient serum antibody level thereby conferring passive protection to individuals with impaired immunity to infections (Barlan, I.B. et al., *J. Allergy, Clin. Immunol.* 92: 353-355, 1993; Buckley, R.H., *JAMA* 258: 2841-2849, 1987). It is possible that IVIG improves asthma by alleviating any persistent infection in the airways that could be etiologic in some asthmatic exacerbations. In addition, therapy with IVIG may provide broadly reacting anti-idiotypic antibodies thereby attenuating hyperreactive responses in asthmatic patients (Busse, W.W. et al., *Am. J. Respir. Crit. Care Med.* 154: S70-S72, 1996). At higher doses, IVIG may act as an immunomodulator to regulate the immune system. IVIG may interrupt the cascade of inflammatory events that underlie asthma and modulate the regulation of IgE antibody production.

Conversely, conflicting reports exist to indicate the null-effect of IVIG therapy in adult or pediatric asthmatic patients (Fontana, V., *J. Pediatr.* 62: 80-84, 1963; Abernathy, R. et al., *Pediatr.* 21: 980-993, 1958). A more recent study by Jakobsson, T. et al. (*Allergy* 49:

413-420, 1994) revealed that the benefits of IVIG in moderately severe asthmatic patients were only slight and transient and the investigators did not recommend the general use of IVIG in asthma. To date, the role of IVIG in asthma therapy is reserved as an experimental-type alternative agent (Montanaro, A., *J. Asthma* 31: 227-229, 1994). This may be attributed, in part, to its relatively high cost, the invasiveness of therapy, and unproven efficacy compared to existing commercially available anti-asthmatic agents.

An increasing understanding of the pathogenesis of asthma has led to the implication of diverse immune mediators in asthma etiology which have become the targets for anti-asthmatic immunotherapy. Specific immune globulin products targeting these mediators have been designed and tested, examples of which are listed as follows.

United States Patent Nos. 5,449,760 and 5,342,924 and PCT Patent Application, published as WO 97/33616 describe a humanized murine anti-IgE antibody and its therapeutic potential in treating allergic diseases including asthma. Such anti-IgE antibodies are also documented previously by Kolbinger, F. et al. (*Protein Eng.* 6: 971-980, 1993; Kolbinger, F. et al., *Biotechnol.* 94-112: 49-51, 1994).

Zhou, C.Y. et al. (*J. Asthma* 34: 195-201, 1997) demonstrated the ability of anti- interleukin-4 antibodies to inhibit IgE production and proposed their usefulness in the prophylaxis of asthma.

United States Patent No. 5,670,626 describes monoclonal IgA antibodies specific against major allergenic proteins found in ragweed, dust mites and animal dander. These antibodies are potentially useful in recognizing and neutralizing major allergens associated with the etiology of extrinsic asthma.

European Patent Publication Nos. 528,931, 528,951 and 551,501 and United States Patent No. 5,324,510 describe humanized IgG antibodies specific against intercellular adhesion molecule (ICAM)-1, endothelial-leucocyte adhesion molecule (ELAM)-1 or CD18 which are implicated in the mediation of airway abnormalities in asthma. Interference of the functions of adhesion molecules is a plausible approach for asthma therapy.

Other mediator-specific immune globulins that are potentially useful in asthma are anti-CD4 antibodies targeting T-lymphocytes (PCT Patent Application, published as WO 92/08474); anti-endothelin antibodies for the treatment of vasoconstrictive illnesses (European Patent publication No. 384,144); humanized antibodies specific against alpha-4 integrin (PCT Patent Application, published as WO 97/18838; Lobb, R.R. et al., *Eur. Respir. J.* 9 Suppl. 22: 104s-108s, 1996); antibodies specific against platelet activating factor (PAF) (European Patent publication No. 761,232); and humanized anti-VLA-4 IgG or IgA antibodies (European Patent publication Nos. 678,122 and 626,861).

SUMMARY OF THE INVENTION

The present inventor has surprisingly found that antibodies specific against Rh antigens are effective in the prophylaxis and treatment of asthma in mammals with or

without immunodeficiency. These Rh antibodies and their use are directed toward antigenic targets that are clearly different than those recognized by antibodies of the prior art.

The present inventor has found that Rh antibodies are useful for the preventing and treating type I hypersensitivity disorders. Accordingly, broadly stated, the present invention relates to a method for preventing and treating asthmatic exacerbations or episodes in an animal comprising administering an effective amount of Rh antibodies or anti-Rh immunoglobulin to an animal in need thereof. In one embodiment, the type I hypersensitivity disorder is asthma. In preferred embodiments, an Rh positive human subject is treated with anti-Rh_o(D) immunoglobulin and an Rh negative subject is treated with anti-c immunoglobulin.

In another aspect of the invention, a process is provided for preparing an anti-Rh immunoglobulin fraction comprising contacting an aqueous animal plasma fraction containing IgG with two different chromatographic separation columns to produce a purified IgG-rich fraction; and treating said purified IgG fraction with a solvent/detergent process.

In a further aspect, the present invention also relates to a pharmaceutical composition for use in preventing and treating a type I hypersensitivity disorder comprising Rh antibodies or anti-Rh immunoglobulin in admixture with a suitable diluent or carrier. In one embodiment, the composition for preventing the onset and reducing the severity or duration of asthmatic exacerbations comprises an anti-Rh immunoglobulin, preferably human anti-Rh immunoglobulin, in admixture with a suitable diluent or carrier.

In yet another aspect, the invention provides the use of anti-Rh immunoglobulin for preventing or treating a type I hypersensitivity disorder, such as asthmatic exacerbations or episodes.

In a further aspect, the present invention provides the use of anti-Rh immunoglobulin for manufacturing a medicament for preventing or treating a type I hypersensitivity disorder such as anti-asthma therapy or prophylaxis.

Other features and advantages of the present invention will become apparent from the following detailed description and attached drawings. In addition, reference is made herein to various publications, patents and patent applications which are hereby incorporated by reference in their entirety. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a treatment schedule for WinRho SD® in asthma and the efficacy of WinRho SD® in preventing asthmatic episodes.

Figure 2 illustrates the efficacy of WinRho SD® in decreasing patient

granulocyte and monocyte counts.

Figure 3 illustrates the efficacy of WinRho SD® in reducing the immune responsiveness of Peripheral Blood Mononuclear Cells.

DETAILED DESCRIPTION OF THE INVENTION

As hereinbefore mentioned, the present inventor has found that Rh antibodies, in particular anti-Rh_0(D) immunoglobulin, are useful in preventing and treating type I immediate hypersensitivity reactions including asthmatic exacerbations. Accordingly, broadly stated the present invention relates to a method for preventing and treating a type I hypersensitivity disorder in an animal comprising administering an effective amount of Rh antibodies or anti-Rh immunoglobulin to an animal in need thereof. In one embodiment, the type I hypersensitivity disorder is asthma.

The term "effective amount" means an amount effective, at dosages and for periods of time necessary to achieve the desired result.

The term "animal" means all members of the animal kingdom including humans.

The term "Rh antibodies" means antibodies specific for antigens of the Rh blood group system, or epitopes thereof (see Rh Blood Group System, in Blood Transfusion in Clinical Medicine, ed. Mollison, P.L. et al., chapter 8, page 328, for a review of the Rh blood group antigens, which is incorporated in its entirety herein by reference). Examples of the Rh antibodies include anti-D (also known as anti-Rh_0, and also referred to herein as anti-Rh_0(D)); anti-C (also known as anti-rh'); anti-E (also known as anti-rh''); anti-c (also known as anti-hr') and anti-e (also known as anti-hr''). The Rh antibodies of the present invention may be preparations from plasma enriched for Rh antibodies, polyclonal antibodies, monoclonal antibodies, antibody fragments (e.g. Fab, and $F(ab')_2$), and those produced by recombinant DNA technology.

In one embodiment, the invention provides a method for treating or preventing asthma comprising administering an effective amount of Rh antibodies to an animal in need thereof. In particular, the present inventor has found that asthmatic patients who have been treated with anti-Rh_0(D) immunoglobulin suffer from fewer and less severe asthmatic exacerbations. In patients with more severe form of the disease or corticosteroid-dependent asthma, lower corticosteroid dosages are required for disease control when said patient are treated with anti-Rh_0(D) immunoglobulin. While not wishing to be bound by a particular theory, the Rh antibodies act by modulating the body's immune system such as the attenuation of inappropriate immune cell function and the cytokine cascade system. For example, an inhibition of the cytokine and chemokine cascades would reduce the influx of inflammatory cells into the brochial tissue thereby reducing brochoconstriction, mucus secretion and tissue inflammation.

Generally Rh antibodies for use in the invention are selected depending on the Rh antigens present/absent on the red cells of the subject to be treated. Anti-Rh_0(D) is preferably used to treat Rh-positive (i.e. D-positive) subjects, and anti-c is preferably used to treat Rh-negative (i.e. D-negative) subjects.

Preparations with a high Rh antibody content may be isolated as an immune globulin fraction from plasma, preferably human plasma, using conventional techniques. For example, they may be isolated using: (a) the Cohn cold ethanol fractionation method or modifications thereto (see Huchet, J. et al., *Rev. Fr. Transfus.* 13:231, 1970; Chown, B. et al., *Can. Med. Assoc. J.* 100:1021, 1969; Jouvenceaux, A. et al., *Rev. Fr. Transfus.* 12 (suppl.): 341, 1969; Barandun, S. et al., *Vox Sang.* 7: 157-174, 1962); (b) ion-exchange chromatographic methods (*e.g.* using DEAE-Sephadex) and modifications thereto may be used to produce Rh antibodies of higher product yield and quality (Cunningham, C.J. et al., *Biochem. Soc. Trans.* 8: 178, 1980; Hoppe, H.H. et al., *Vox. Sang.* 25: 308, 1973; Hoppe, H.H. et al., *Münch. Med. Wochenschr.* 109: 1749, 1967; Baumstark, J.S. et al., *Arch. Biochem.* 108:514, 1964); or (c) anion-exchange chromatographic method as taught in Canadian Patent No. 1,201,063, and modifications thereto. Commercially available anti-Rh_0D immune globulin preparations may also be used in the methods. For example, anti-Rh_0D preparations including WinRho® or WinRho SD® (Cangene Corporation), HypRho-D® (Miles Canada Inc.) or RhoGAM® or MICRhoGAM® (Ortho Diagnostics) may be used in the present invention.

In an embodiment of the invention, an anti-Rh_0D immune globulin fraction is prepared by contacting an aqueous plasma fraction containing IgG with one or more chromatographic separation columns to produce a purified IgG-rich fraction. The aqueous plasma fraction used in the process may be normal non-immunized plasma from an animal source, preferably a human source, or hyperimmune plasma such as plasma from Rh alloimmunized donors. For example, the Rh_0D antigen is used to immunize the animal through intramuscular, subcutaneous, intraperitoneal, or intraocular injection, with or without an adjuvant such as Freund's complete or incomplete adjuvant. With the option of booster immunizations, samples of serum are collected and tested for reactivity to the antigen in standard assays (described below). Particularly preferred polyclonal antisera will give a signal on one of the assays that is at least three times greater than background. Once the titre of the animal has reached a plateau in terms of antigen reactivity, larger quantities of the antisera may be obtained readily either by periodic bleeding or by exsanguinating the animal.

Human anti-Rh_0D immune globulin may also be produced in human volunteers. For example, an anti-Rh_0D immune globulin preparation may be obtained from a subject who is naturally immunized (*e.g.* from an Rh incompatible pregnancy) or artificially immunized using Rh-positive blood cells or Rh_0D antigen.

Anti-Rh_0D immune globulin-containing plasma collected from animal or human is modified to the ionic strength and pH of the initial buffer used with the chromatographic separation column. In an embodiment of the invention, the aqueous animal plasma fraction is contacted with one or more, preferably one to two, anionic exchangers to produce a purified IgG-rich fraction.

By way of example, aqueous animal or human plasma fraction is applied to an anion exchange column which may contain an agarose cross-linked anionic exchange resin such as DEAE-Sepharose CL6B or DEAE-Biogel, and an IgG-rich fraction is obtained by eluting with an equilibrating buffer. The IgG-rich fraction may be concentrated for example by ultrafiltration. The concentrated IgG-rich fraction is then applied to a second different anion exchange column such as DEAE-Biogel or DEAE-Sephadex A-50. A purified IgG-rich fraction is isolated by elution with an appropriate equilibrating buffer which may be further purified using ultrafiltration.

The purified IgG protein may optionally be treated with a solvent and detergent to inactivate lipid envelope viruses. Suitable solvents and detergents which may be used include Triton X-100 and tri(n-butyl) phosphate (Horowitz, B., *Curr. Stud. Hematol. Blood Transfus.* 56: 83-96, 1989). After the process, said solvents and detergents may be removed using conventional methods such as reverse phase chromatography.

Monoclonal immune globulins may also be produced readily using recombinant and hybridoma techniques (see Canadian Patent number 1,303,534; Canadian Patent number 1,303,533; European Patent Application 87302620.7 published as EP 239,400; European Patent Application 93102609.0 published as EP 557,897; Fletcher, A. and Thompson, A., *Transfus. Med. Rev.* 9: 314-326, 1995; Alting-Mees, M. et al., *Strat. Mol. Biol.* 3: 1-9, 1990; Huse, W.D. et al., *Science* 246: 1275-1281, 1989; Sastry, L. et al., *Proc. Natl. Acad. Sci. USA* 86: 5728-5732, 1989). Similarly, binding partners or domains may be constructed using recombinant DNA techniques to incorporate the variable regions of a gene encoding a specific antibody (see PCT Patent Application PCT/GB93/00605 published as WO 93/19172; PCT Patent Application PCT/GB93/02492 published as WO 94/13804; PCT Patent Application PCT/EP90/01964 published as WO 91/07492; Bird et al., *Science* 242: 423-426, 1988). It will be apparent to one skilled in the art that the fractionation and recombinant approaches may be applied to diverse types of immune globulins. For example, specific monoclonal immune globulins against different antigens may be generated by techniques based on the same principle of recombinant DNA technology.

Anti-Rh immunoglobulin produced by the processes above may be formulated with a wetting agent (non-ionic surface active agents) such as polysorbate 80, also known as TWEEN 80®. The immune globulin preferably is at least about 95% pure, more preferably about 99.5% pure and the wetting agent reduces the amount of fragmentation over extended periods of time to provide a highly stable preparation enriched for Rh antibodies. Non-ionic

surface agents such as sorbitan esters or polyoxyethylene sorbitan esters of fatty acids (TWEEN® or SPAN® type surface active agents) may be prepared by methods well known in the art and added to a final concentration of about 0.005% to about 0.5%. Said surface active agents may also be obtained commercially from J.T. Baker Inc. (Phillipsburg, New Jersey, USA), ICI Atkemix (Brantford, Ontario, Canada), Van Waters and Rogers Ltd. (Richmond, British Columbia, Canada), or Nikkol Co. (Japan). Other stabilizers such as sodium chloride (final concentration of up to about 0.9%), mannitol, and/or L-glycine or L-histidine (final concentration of about 0.025M to 0.05M) can also be used to achieve or further stabilization of such anti-Rh immunoglobulin preparation, and the pH of the fraction may be adjusted within the range of 4.0 to 5.4. The resulting preparation may be sterilized, for example, by filtration. If desired the preparation may be freeze-dried, and reconstituted using a suitable solution such as sterile Water For Injection (USP) or 0.9% sodium chloride.

By way of example and reference, currently available intravenously injectable immune globulin preparations commonly consist of an immune globulin distributed in a physiologically compatible medium. Said medium may be sterile water for injection (WFI) with or without isotonic amounts of sodium chloride. For example, the recommended diluent for reconstitution of Iveegam®, Gammagard®, or, Venoglobulin®, is sterile WFI. Sandoglobulin® is supplied with 0.9% (w/v) sodium chloride solution as diluent (see Gahart, B.L. & Nazareno, A.R., Intravenous Medications: a handbook for nurses and allied health professionals, p. 516-521, Mosby, 1997). WinRho SD®, an anti-Rh_0D immune globulin produced by Cangene Corporation, is reconstituted in 0.9% sodium chloride solution for intravenous injection. The immune globulin product by Schura (cited above) is formulated as a solution of 165 mEq/L sodium ion and 120 mEq/L chloride ion with a final pH of 6.7. The Miles' intravenous immune globulin preparation, Gammimune®, when constituted, has an osmolality of 278 mOsm/L and a pH of 4.0-4.5. U.S. patent Nos. 4,396,608 and 4,499,073 also disclose a low pH (3.5-5.0) and low ionic strength ($\leq$0.001) immune globulin formulation for intravenous injection. The globulin protein concentration in the above preparations ranges from 0.5% to 20%.

A preferred preparation obtained using the processes described above for the present invention has the following characteristics: 2-3% human immunoglobulin, no or very low level buffer, essentially no ionic strength, 10 ppm polysorbate 80 and 10% sorbitol, pH 4.0.

It will be apparent to one skilled in the art that the preparations used in the present invention may contain more than one type of Rh antibody. For example, a preparation may contain both anti-Rh_0(D) and anti-c. Such compositions are for intravenous, intramuscular, subcutaneous, oral, enteral, intranasal, intrapulmonary or inhalational use. In particular, those forms for intramuscular or subcutaneous administration are used, or forms for infusion or intravenous injection are used, which can be prepared as solutions of the antibodies

or as powders of the antibodies to be mixed with one or more pharmaceutically acceptable excipients or diluents, suitable for the aforesaid uses and with an osmolarity which is compatible with the physiological fluids. For example, as described herein, an Rh antibody preparation may be formulated with a wetting agent and/or stabilized by addition of a stabilizer. Preferably, the preparations are in a form suitable for intravenous or intramuscular administration (e.g. WinRho SD® and WinRho SDF™, Cangene Corporation, Winnipeg, Canada). When administering the compositions/preparations of the invention by injection, the administration may be by continuous infusion or by single or multiple bolus injections.

In an embodiment of the invention, forms for intravenous injection or infusion are selected to maximize drug bioavailability, reduce dosage, and to elicit faster pharmacodynamic action. For example, Rh negative subjects were injected with adult and fetal Rh-positive red blood cells and subsequently WinRho SD® (e.g. 120 ug) was administered by intravenous or intramuscular injection. Peak plasma levels of WinRho SD® were achieved immediately after intravenous injection but were only achieved 24 after intramuscular injection. Intravenous injection also produced two-fold higher peak plasma levels than intramuscular injection. Clearance of Rh-positive red blood cells was complete within 8 hours of intravenous administration, and 24 hours of intramuscular administration (Bowman, J.M. et al., *CMA Journal* 123: 1121-1125, 1980). In the present invention, a faster red blood cell clearance may correlate with a faster onset of action.

The compositions of the invention may contain one or more Rh antibodies together with one or more other active substances. Examples of active substances which may be used in the compositions/preparations include bronchodilators such as beta-adrenergic receptor agonists (albuterol, terbutaline, pirbuterol, salbutamol, salmeterol, formoterol) and xanthine derivatives (theophylline, enprofylline, pentoxifylline), anti-inflammatory agents such as cromolyn-like drugs (cromolyn sodium, nedocromil) and glucocorticoids (prednisone, methylprednisolone sodium succinate, beclomethasone diproprionate, triamcinolone acetonide, flunisolide, budesonide diproprionate, fluticasone proprionate), and miscellaneous therapeutics such as troleandomycin, methotrexate, gold, hydroxychloroquine, dapsone and cyclosporine A. The Rh antibodies and active substances may be administered by any conventional means available for the use in conjunction with pharmaceuticals, either as individual separate dosage units administered simultaneously or concurrently, or in physical combination of each component in a single or combined dosage unit. The Rh antibodies and active substances can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice as described herein.

The combination of Rh antibodies and active substances may result in a synergistic action which enhances the effects of the Rh antibodies, or enhances the effects of

the active substances. The doses of Rh antibodies and active substances may be each selected so that the Rh antibodies and active substances alone would not show a full effect.

The compositions of the invention can be intended for administration to human or animals. An appropriate preparation may be selected for a particular subject based on the presence/absence of Rh antigens on the surface of the red blood cells of the subject. Said compositions/preparations are intended to provide to the recipient subjects an amount of Rh antibodies sufficient to prevent or treat asthmatic exacerbations. More specifically, the amount is said to be sufficient if the dosage, route of administration, etc., of the Rh antibodies are sufficient to reduce the intensity, duration and/or frequency of asthmatic exacerbations or if the required dosages of other medications that the recipient subject may receive can be reduced without compromising therapeutic effectiveness.

Dosages of anti-Rh_0D immune globulin in the formulations of the present invention depend on individual needs, on the desired effect in a particular therapeutic indication, and on the chosen route of drug administration. Daily dosages to humans by intramuscular or intravenous injection generally vary between about 10 ug (50 IU) to 400 (2,000 IU) ug per kg body weight. For intramuscular injection, the preferred dosage is about 20 ug (100 IU) to 400 ug (2,000 IU) per kg body weight. For intravenous injection, the preferred dosage is about 10 ug (50 IU) to 200 ug (1,000 IU) per kg body weight, preferably 50 ug (250 IU) per kg body weight. These dosages are significantly lower than those suggested for intravenous immune globulin (IVIG) treatment of asthmatic patients. The lower dosages provided in the present invention also reduce the risk of adverse reactions such as cardiovascular and thromboembolic events.

The compositions and preparations described herein can be prepared by *per se* known methods for preparing pharmaceutically acceptable compositions which can be administered to human subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for instance, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, USA, 1985). On this basis, the compositions include, albeit not exclusively, solutions of Rh antibodies in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and isoosmotic with the physiological fluids.

Pharmaceutical techniques may also be employed to control the duration of action of the compositions/preparations of the present invention. Control release preparations may be prepared through the use of polymers to complex, encapsulate, or absorb the Rh antibodies.

The therapeutic effects of the present invention may be obtained by providing to a patient any of the above described Rh antibody compositions or preparations. The compositions and preparations may be provided to patients who are suffering from, or are

susceptible to, episodes of asthmatic exacerbations. The compositions and preparations may also be provided to patients who are undergoing therapy with another mode of asthma intervention such as corticosteroid therapy.

Having generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

EXAMPLE 1

Rh Antibodies Alleviate Asthmatic Exacerbations

This example illustrates a schedule for treating asthmatic patients and preventing asthmatic exacerbations. Rh_0(D)-positive asthmatic patients are treated with multiple courses of intravenous WinRho SD® (Cangene's brand of anti-Rh_0D immunoglobulin). The courses are given at 3-week intervals, and each course consists of intravenous injections of about 25 to 50 microgram (ug) per kilogram (kg) per day WinRho SD® for 2 consecutive days for a total of about 50 to 100 ug per kg per course. The total dose of WinRho SD® may be combined and given on a single occasion, and throughout the treatment period, the dosage of WinRho SD® may be adjusted according to the individual patient's clinical response. Efficacy of WinRho SD® treatment may be monitored by:

1. monitoring asthmatic symptoms in patients and the number of exacerbations or episodes per time period;
2. assessing patient symptom scores (0, no symptom; 1, mild symptoms but no discomfort; 2, moderate symptoms with no change in daily routine; 3, severe symptoms interfering with sleep or activity; 4, very severe incapacitating symptoms);
3. assessing patient pulmonary function by spirometry and body plethysmography to measure lung volume, resistance, specific conductance and flow rates;
4. performing skin prick testing (SPT) using different dilutions of commercial antigens (Greer Laboratories, N.Y. or Hollister-Stier Laboratories, Utah) to assess patient immune reactivity with histamine as positive control.

Intravenous WinRho SD® therapy at this dosage should reduce the frequency, intensity and duration of asthmatic exacerbations in asthmatic patients. Patient symptom score (e.g. average monthly symptom score) and pulmonary function (e.g. lung volumes, flow rates such as FEV_1 and FVC) should improve with therapy and a progressive diminution in skin test reactivity (increase in tolerance to higher antigen concentrations and decrease in end point titration) should become evident within 6 months of WinRho SD® therapy.

EXAMPLE 2

Corticosteroid-Sparing Effect of Rh Antibodies

This example illustrates the synergistic utility of anti-Rh_0D immunoglobulin

with other anti-asthma therapeutic agents. WinRho SD® is administered intravenously to patients receiving glucocorticoid therapy. Dosage regimentation of intravenous WinRho SD® is substantially as described in Example 1. Effective therapeutic dosages of glucocorticoids may constitute an average oral dose of 1 to 2 mg per kg patient body weight of prednisone on alternate days. Alternatively, an effective inhalational dose of a glucocorticoid is 0.4 mg to 0.8 mg beclomethasone diproprionate per day administered via a metered dose inhaler.

Combinational therapy of a glucocorticoid with anti-Rh_0D immunoglobulin should provide greater and more sustained effect in preventing and treating asthmatic exacerbations than monotherapy. Consequently, escalation of glucocorticoid dosage (commonly required in more severe asthmatic patients) could be avoided and the maintenance dosage of the glucocorticoid (*e.g.* average daily, weekly or monthly dose of glucocorticoid) could be reduced significantly within 6 months of WinRho SD® therapy so to minimize glucocorticoid-associated side effects.

EXAMPLE 3

WinRho SD® in Prevention of Asthmatic Episodes

A female Rh_0(D)-positive patient of 38 years of age presented with a history of mild to moderate asthma for over 3 years was treated with WinRho SD® in 1996. The patient received repeated courses of intravenously administered WinRho SD® at about 50 to 100 ug per kg body weight every two to three weeks for 14 weeks. The exact treatment schedule is presented Figure 1. All adverse events were monitored and recorded during and after the treatment period.

During the entire duration of the WinRho SD® treatment period (for over 100 days), the patient did not experience any asthmatic episodes. Conversely, upon cessation of WinRho SD® therapy, recurrence of asthma was evidenced by two asthmatic episodes reported on Days 183 and 225. The patient was again treated with WinRho SD® on Days 225 and 280 and all symptoms of asthmatic episodes subsided thereafter. The present crossover data clearly indicate the ability of anti-Rh_0D immunoglobulin in preventing the onset of and reducing the frequency of asthmatic episodes.

EXAMPLE 4

Corticosteroid-Sparing effects of WinRho SD® in Asthma

A second Rh_0(D)-positive asthmatic male patient of 53 years of age who is HIV-positive and has significant lymphopenia was treated with WinRho SD®. This patient has a long history of asthma of over 44 years received intermittent WinRho SD® treatment over a period of 6 months in 1992. Approximately 50 ug per kg patient weight (3000 ug) WinRho SD® was administered to the patient on days 1, 17, 25, 96, 145 and 173 of the study, and all adverse events and concurrent medications were monitored and recorded throughout the study.

During the entire course of this 6-month study, this patient experienced only 2 asthmatic episodes and required 10 mg oral prednisone on the each occasion. The intensity of corticosteroid intervention for asthma control observed during the WinRho SD® treatment period was significantly lower than the 1 to 2 mg prednisone per kg patient body weight per day conventionally required. The 10 mg dosages of prednisone administered to the patient are equivalent to about 0.2 mg per kg patient body weight and represent only 10-20% of the recommended dosage of prednisone for asthma control.

EXAMPLE 5

WinRho Decreases Granulocyte and Monocyte Counts

Airway hyperresponsiveness and clinical asthma are at least in part caused by the inflammation generated by cell mediated immune mechanisms involving T cells, monocytes, granulocytes and mast cells. This example illustrates the ability of anti-Rh_o(D) immunoglobulin (WinRho SD®, Cangene Corporation, Winnipeg, Canada) to decrease the number of circulating granulocytes and monocytes in Rh-positive patients *in vivo* and thereby reduce cell-mediated immune hyperreactivity.

In a crossover study, 5 patients were randomized to receive 25 ug/kg or 50 ug/kg WinRho SD® as a single dose by intravenous infusion in the first cycle of treatment. All patients were examined prior to drug administration on day 1 and subsequently at 1 and 7 days after drug administration (days 2 and 8). Subsequently, 3 further cycles of WinRho SD® treatment were given to the patients at alternating high and low dosage regimens for a total of 4 courses (*i.e.* 25 ug/kg - 50 ug/kg - 25 ug/kg - 50 ug/kg or 50 ug/kg - 25 ug/kg - 50 ug/kg - 25 ug/kg).

At the time of examination, venous blood samples (5 mL each) were collected by venipuncture into Vacutainer® tubes containing sodium heparin (15 U/mL) as anticoagulant. Peripheral blood smears stained and spread by routine technique (using Wright's-Giemsa stain and a centrifugal peripheral smear device) were prepared in duplicates from the collected blood and were examined visually for granulocyte and monocyte content by conventional microscopic methods. It should be readily apparent to a person skilled in the art that the differential white blood cell count may also be performed using automated instrumentation such as the Coulter S-Plus IV differential cell counter (Coulter Electronics, Hialeah, FL) (Cox, C.J. et al., *Am. J. Clin. Pathol.* 84: 297-306, 1985) or an optical flow cytometer (Hellma GmbH & Co., Mullheim/Baden, Federal Republic of Germany) (Terstappen, L.W. et al., *Cytometry* 9: 39-43, 1988).

Administration of WinRho SD® at 25 ug/kg or 50 ug/kg rapidly reduced the number of circulating granulocytes and monocytes (day 2 data) and a statistically significant reduction at the level of $p < 0.05$ was observed by day 8 (Figure 2). Upon cessation of WinRho SD® therapy, patient granulocyte and monocyte counts returned to baseline levels. Repeated

administration of WinRho SD® at 25 ug/kg or 50 ug/kg in subsequent treatment cycles produced similar dramatic decreases in patient granulocyte and monocyte counts which again reached statistically significant levels by day 8 (course 3 data shown).

The present study provides empirical evidence to demonstrate that anti-Rh_0(D) immunoglobulin significantly reduces the number of cells that are involved in the immune hyper-responsiveness in asthma and other type I hypersensitivity disorders. In this respect, anti-Rh_0(D) immunoglobulin is useful for the correction of the exaggerated smooth muscle dysfunction and profound inflammation by intervening at the mechanistic level.

EXAMPLE 6

WinRho Inhibits Immune Responsiveness of Monocytes and Lymphocytes

This example illustrates further effects of anti-Rh_0(D) immunoglobulin on immune cell function. Type I hypersensitivity reactions occurs when antigenic material which is not in itself noxious (*e.g.* grass pollen, certain foodstuff and drugs) evokes an exaggerated immune response resulting in profound inflammation. Airway hyperresponsiveness to antigen and bronchial inflammation are the major symptoms of clinical asthma.

An antigen-induced immune reaction is mediated through a cascade of cooperative events and a foreign antigen alone is not sufficient to elicit a full scale immune response. Activation of naive T-lymphocytes requires a co-stimulatory signal delivered by a professional antigen presenting cell (APC) and the three major types of APCs are B-lymphocytes, macrophages and dendritic cells. Upon recognition of an antigen, the APC internalizes, processes and displays the antigen (or fragments thereof) on an appropriate major histocompatibility complex (MHC) with a co-stimulatory molecule on the cell surface.

Once the antigen-MHC is presented to the T-lymphocyte receptor, the T-lymphocyte becomes activated and is able to elicit both cell-mediated and humoral immune responses including the activation of macrophages to effect lysozyme fusion and the activation of B-lymphocytes to produce specific antibodies against the antigen. The primary means by which T- lymphocytes exert their actions is through the secretion of cytokines such as interleukin (IL-2). Secreted interleukin-2 (IL-2) also promotes T-cell clonal expansion and differentiation and cause excessive infiltration and upregulation of the function of eosinophils and mast cells. In asthma, the excessive release of cytokines and the IgE type antibodies produced by activated B-lymphocytes both stimulate masts cells to secrete histamine to produce bronchial inflammation.

In the crossover study of Example 5, also illustrated is the ability of anti-Rh_0(D) immunoglobulin (WinRho SD®) to suppress the responsiveness of immune cells and to reduce the magnitude of immune response mounted against a foreign antigen. To

reiterate, 5 patients were randomized to receive 25 ug/kg or 50 ug/kg WinRho SD® as a single dose by intravenous infusion in the first cycle of treatment. All patients were examined prior to drug administration on day 1 and subsequently at 1 and 7 days after drug administration (days 2 and 8). Subsequently, 3 further cycles of WinRho SD® treatment were given to the patients at alternating high and low dosage regimens for a total of 4 courses.

At the time of examination, venous blood samples (10 mL each) were collected by venipuncture into Vacutainer® tubes containing sodium heparin (15 U/mL) as anticoagulant. Peripheral blood mononuclear cells (PBMCs) were obtained from the collected blood by standard techniques using centrifugation (1,200*g* for 30 minutes at 20°C) through a 1.077 g/mL Percoll (Pharmacia LKB, Baie d'Urfe, Quebec, Canada) gradient (Semple, J.W. and Freedman, J., *Blood* 78: 2619-2625, 1991).

The resulting cell sample contained approximately 18% DR^+ antigen-presenting cells (APC) (9% $CD19^+$ B cells and 9% $CD14^+$ monocytes/macrophages), approximately 55% T cells and 25% $CD3^-CD56^+$ natural killer (NK) cells. T lymphocyte and monocyte enrichment was optionally performed using a modified method of Gutierrez et al. (*J. Immunol. Methods* 29: 57, 1979). Briefly, the washed PBMCs were layered onto a discontinuous Percoll gradient consisting of the following densities: 1.091 g/mL (70%), 1.060 g/mL (50%), 1.050 g/mL (40%), and 1.030 g/mL (30%); and then centrifuged at 1,200*g* for 30 minutes at 20°C. The PBMC band at the 30%/40% Percoll interface were enriched for DR^+ APCs [approximately 46% DR^+ APC (14% $CD19^+$ and 32% $CD14^+$)] while the band at the 50%/70% Percoll interface were enriched for T cells [approximately 65% T-lymphocytes]. All *in vitro* assays were performed in RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) containing 5% pooled inert human group AB serum, 2 mmol/L L-glutamine (GIBCO), 100 g/mL penicillin/streptomycin (GIBCO), and 5×10^{-5} mol/L 2-mercaptoethanol (cRPMI).

Patient immune cell responsiveness was examined by a seven-day PBMC assay using allogeneic platelets as stimulus (Semple, J.W. et al., *Blood* 78: 2619-2625, 1991; Semple, J.W. et al., *Blood* 78: 474, 1991). Enriched DR^+ APC and T-cell enriched PBMCs (4:1 T:DR^+ ratio) were cultured in 96-well round-bottom tissue culture plates in cRPMI. Allogeneic platelets were prepared by conventional methods (Semple, J. et al., *Blood* 87: 4245-4254, 1996; Freedman, J. and Hornstein, A., *Am. J. Hematol.* 38: 414, 1991) and were titrated into the cultures (about 2×10^5 PBMC) and incubated for 6 days at 37°C. On day 6, 50 L of culture medium was removed from each well and tested for IL-2 content by stimulation of proliferation of a murine IL-2-dependant cell line, CTLL (Semple, J.W. et al., *Blood* 86: 805-812, 1995; Semple, J.W. et al., *Blood* 78: 474, 1991). ^{3}[H]-thymidine (1 uCi) was then added to each well, and the plates were incubated for 24 hours at 37°C. The cells were then harvested with a Skatron cell harvester onto filter discs and incorporated radioactivity (reflecting proliferative activity) was counted in an LKB Rack Beta counter. PBMC

supernatant IL-2 levels were calculated based on recombinant human IL-2 standards (GIBCO-BRL, Gaithersburg, MD).

Human allogeneic platelets showed no *in vitro* stimulatory activity on T-cell enriched cultures without DR^+ APCs. However, the addition of allogeneic platelets to DR^+ APC and T- cell enriched culture (collected prior to WinRho SD® therapy first cycle) demonstrated significant immune reaction as evidenced by the increase in CTLL cell proliferation caused by the high levels of IL-2 secreted by APC-activated T-lymphocytes into the PBMC culture medium (Figure 3). Treatment of the patient with 25 ug/kg or 50 ug/kg WinRho SD® dramatically inhibited T-lymphocyte activation by the antigenic stimulus and the level of IL-2 secretion by T-lymphocytes in culture decreased virtually to baseline level by day 8 after the first treatment. Surprisingly, the effect of WinRho SD® was sustained beyond day 8 and the inhibition of IL-2 release remained apparent at the time of the second treatment. Thereafter, the level of IL-2 secretion remained at baseline throughout the subsequent 3 cycles of therapy.

The present study provides further evidence to support the view that anti-Rh_o(D) immunoglobulin is useful for correcting the exaggerated immune response underlying type I hypersensitivity disorders by significantly reducing the inherent immune responsiveness of T-lymphocytes, through APC mediation, to an artificial antigenic stimulus. Inhibition of cytokine release by T-lymphocytes would in turn prevent downstream cell-mediated and humoral immune responses that cause tissue inflammation and other clinical symptoms of a type I hypersensitivity disorder.

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated by those skilled in the art that invention can be modified in arrangement and detail without departure from such principles. We claim all modifications coming within the scope of the following claims.

I CLAIM:

1. A method for treating or preventing a type I hypersensitivity disorder comprising administering an effective amount of Rh antibodies to an animal in need thereof.

2. A method for treating or preventing asthma comprising administering an effective amount of Rh antibodies to an animal in need thereof.

3. A method for reducing the severity of asthmatic exacerbations in an animal comprising administering an amount of Rh antibodies sufficient to reduce the intensity and duration of asthmatic exacerbations to an animal in need thereof.

4. A method for reducing the severity of asthmatic exacerbations in an animal comprising administering an amount of Rh antibodies sufficient to improve pulmonary function during asthmatic exacerbations to an animal in need thereof.

5. A method for preventing asthmatic exacerbations in an animal comprising administering an amount of Rh antibodies sufficient to reduce the frequency of asthmatic exacerbations to an animal in need thereof.

6. A method for preventing asthmatic exacerbations in an animal comprising administering an amount of Rh antibodies sufficient to reduce the required maintenance dosage of a glucocorticoid to an animal in need thereof.

7. A method according to any one of claims 1-6, wherein the animal is Rh positive and the Rh antibodies are anti-Rh_0D immune globulin.

8. A method according to any one of claims 1-6, wherein the subject is Rh negative and the Rh antibodies are anti-c immune globulin.

9. A method according to any one of claims 1-6, wherein the Rh antibodies are polyclonal immune globulin prepared from mammalian plasma or serum.

10. A method according to any one of claims 1-6, wherein the Rh antibodies are monoclonal antibodies or binding partner against the Rh antigen.

11. A method according to any one of claims 1-6, wherein the Rh antibodies are administered by parenteral injection or infusion at a dose of about 10 ug to 400 ug per kg

body weight.

12. A method according to any one f claims 1-6, wherein the Rh antibodies are administered intravenously at a dose of about 10 ug to 200 ug per kg body weight.

13. A method according to any one of claims 1-6, wherein the Rh antibodies are administered intramuscularly at a dose of about 20 ug to 400 ug per kg body weight.

14. A method according to any one of claims 1-6, wherein the Rh antibodies are administered consecutively, simultaneously or in combination with a brochodilator.

15. A method as claimed in claim 14 wherein the brochodilator is a beta-adrenergic receptor agonist.

16. A method as claimed in claim 15 wherein the beta-adrenergic receptor agonist is albuterol, terbutaline, pirbuterol, salbutamol, salmeterol or formoterol.

17. A method as claimed in claim 14 wherein the brochodilator is a xanthine derivative.

18. A method as claimed in claim 17 wherein the xanthine derivative is theophylline, enprofylline or pentoxifylline.

19. A method according to any one of claims 1-6, wherein the Rh antibodies are administered consecutively, simultaneously or in combination with an anti-inflammatory agent.

20. A method as claimed in claim 19 wherein the anti-inflammatory agent is a cromolyn-based compound.

21. A method as claimed in claim 20 wherein the cromolyn-based compound is cromolyn sodium or nedocromil.

22. A method as claimed in claim 19 wherein the anti-inflammatory agent is a glucocorticoid.

23. A method as claimed in claim 22 wherein the glucocorticoid is prednisone, methylprednisolone sodium succinate, beclomethasone diproprionate, triamcinolone acetonide, flunisolide, budesonide diproprionate or fluticasone proprionate.

24. A method according to any one of claims 1-6, wherein the Rh antibodies are administered consecutively, simultaneously or in combination with one or more agents selected from a group consisting of troleandomycin, methotrexate, gold, hydroxychloroquine, dapsone and cyclosporine A.

25. A composition for treating or preventing a type I hypersensitivity disorder comprising an effective amount of Rh antibodies in admixture with a suitable diluent or carrier.

26. A composition for treating or preventing asthma comprising an effective amount of Rh antibodies in admixture with a suitable diluent or carrier.

27. A composition for reducing the severity of asthmatic exacerbations comprising an effective amount of Rh antibodies sufficient to reduce the intensity and duration of asthmatic exacerbations in admixture with a pharmaceutically acceptable carrier.

28. A composition for reducing the severity of asthmatic exacerbations comprising an amount of Rh antibodies sufficient to improve pulmonary function during asthmatic exacerbations in admixture with a pharmaceutically acceptable carrier.

29. A composition for preventing asthmatic exacerbations comprising an amount of Rh antibodies sufficient to reduce the frequency of asthmatic exacerbations in admixture with a pharmaceutically acceptable carrier.

30. A composition for preventing asthmatic exacerbations comprising an amount of Rh antibodies sufficient to reduce the required maintenance dosage of a glucocorticoid in admixture with a pharmaceutically acceptable carrier.

31. A composition according to any one of claims 25-30, wherein the Rh antibodies are anti-Rh_oD immune globulin or anti-c immune globulin.

32. A composition according to any one of claims 25-30, wherein the Rh antibodies are polyclonal immune globulin prepared from mammalian plasma or serum.

33. A composition according to any one of claims 25-30, wherein the Rh antibodies are monoclonal antibodies or binding partner against the Rh antigen.

34. A composition according to any one of claims 25-30, wherein the amount of Rh

antibodies is about 10 ug to 400 ug per kg body weight.

35. A composition according to any one of claims 25-30, whereas the amount of Rh antibodies is in a form suitable for parenteral injection or infusion.

FIGURE 1

Treatment schedule for WinRho SD® in asthma

Day	WinRho SD® Dose	Asthma Reported	Day	WinRho SD® Dose	Asthma Reported
1	75 ug/kg (4800 ug)	No	86	79 ug/kg (5100 ug)	No
2	-	No	92	-	No
9	-	No	100	93.8 ug/kg (6000 ug)	No
14	-	No	106	-	No
22	50 ug/kg (3300 ug)	No	119	-	No
29	-	No	183	-	Yes
37	-	No	225	92.2 ug/kg (6000 ug)	Yes
40	75 ug/kg (4800 ug)	No	232	-	No
48	-	No	245	-	No
56	-	No	247	-	No
57	50 ug/kg (3300 ug)	No	266	-	No
63	-	No	280	91.7 ug/kg (6000 ug)	No
75	80 ug/kg (5100 ug)	No	286	-	No
82	-	No	301	-	No

FIGURE 2

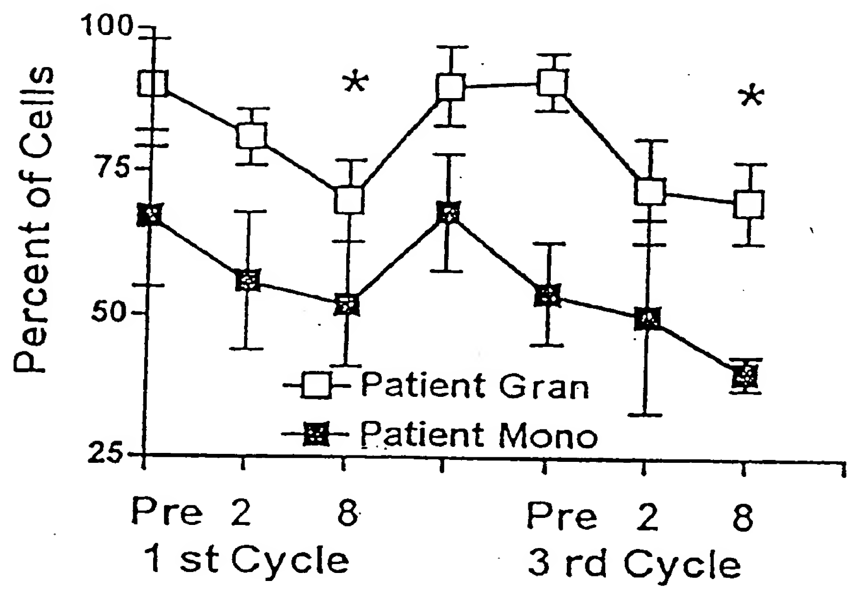
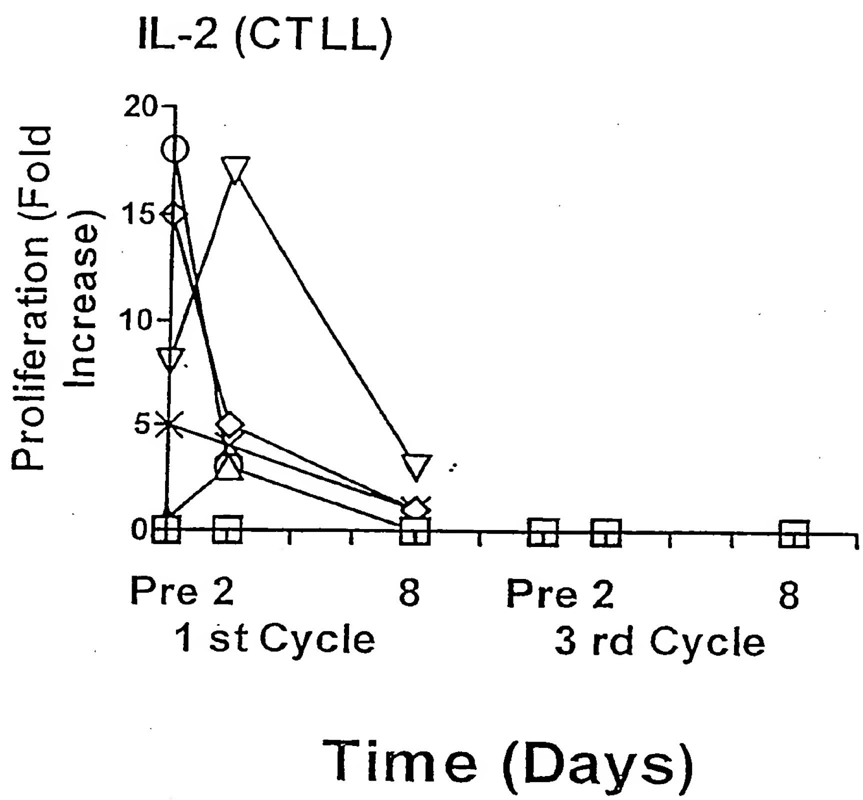

100
75
50
25
Percent of Cells
*
*
Patient Gran
Patient Mono
Pre
2
8
Pre
2
8
1 st Cycle
3 rd Cycle

FIGURE 3


IL-2 (CTLL)
Proliferation (Fold Increase)
20
15
10
5
0
Pre 2
8
Pre 2
8
1 st Cycle
3 rd Cycle
Time (Days)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/00109

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395 A61K38/13 //(A61K39/395,38:13,31:57,31:52,31:44, 31:35,31:165,31:135)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCARADAVOU A. ET AL.: "Intravenous anti-D treatment of immune thrombocytopenic purpura: experience in 272 patients." BLOOD, vol. 89, no. 8, 15 April 1997, pages 2689-700, XP002102874 see abstract	1-35
A	MC CANN M. C. ET AL.: "Production and use of human monolconal anti-D antibodies." JOURNAL OF IMMUNOLOGICAL METHODS, vol. 115, 1988, pages 3-15, XP002102875 see page 11, column 2, line 12 - page 13, column 2, line 29	1-35

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 May 1999	01/06/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Flao, K

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/00109

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	HUGHES-JONES N. C.: "Monoclonal antibodies in haematology." BLOOD REVIEWS, vol. 3, 1989, pages 53-58, XP002102876 see page 57, column 1, line 52 - column 2	1-35
A	WO 96 07740 A (INSTITUT PASTEUR) 14 March 1996 see claim 24	1-35

2

INTERNATIONAL SEARCH REPORT	International application No. PCT/CA 99/00109

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 99/00109

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9607740 A	14-03-1996	FR 2724182 A	08-03-1996
		CA 2198935 A	14-03-1996
		EP 0778891 A	18-06-1997